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Research Article

PREVALENCE AND IDENTIFICATION OF *DEMODEX* MITES IN DOGS IN GUWAHATI, ASSAM OF NORTH-EAST INDIA

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ABSTRACT

Occurrences of skin diseases due to mange mites are responsible for major share of skin diseases in dogs. Demodectic mange, caused by *Demodex* sp. mite is the most common type of mange that occurred in dogs. *Demodex* mite infestation has been reported from several states in India including Assam. However, no systematic study was conducted on molecular detection of the *Demodex* mite in dogs in the North-eastern region of India. Therefore, the present study was designed to explore the prevalence of *Demodex* mite infestation in dogs and also its molecular detection in and around Guwahati, Assam, India from March 2019 to February 2020. Skin scraping was taken from a total of 582 dogs of different breeds, age groups, sex and different categories to study the prevalence and molecular identification of *Demodex* mite. The overall prevalence of *Demodex* mite infestation was recorded to be 19.75% in the present study. The highest prevalence was recorded in pre-monsoon season. The breed, sex, age and category wise study showed the highest prevalence of *Demodex* infestation in Labrador retriever breed, in male dogs, in dogs of below 1 year of age and in stray dogs, respectively. Morphometrically, three species of *Demodex* namely *Demodex injai*, *D. canis* and *D. cornei* could be recorded. To best of our knowledge, this is the first report on molecular detection of *Demodex* sp. in dogs from the North east region of India.

Keywords: 16S rDNA, *Demodex*, Dog, Micrometry, Skin scraping

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Dogs are considered as the most common companion animals of human being. As dogs are the most loved companion animal to humans, their health and well-being is of great importance to their owners. Skin diseases are a very common problem observed in dogs and it has gradually become critical and challenging burden not only for the pet owners but also for the clinicians. The ecto-parasites are the major causes of skin diseases and are usually caused by ticks, mites, fleas, lice, flies etc. They are responsible for causing anaemia, discomfort, dermatitis, hypersensitivity etc. which directly or indirectly affect the health of the dog. Of the ecto-parasites, mange accounts for a major share of skin diseases in dogs causing dermatitis, pruritis, irritation, self-inflicting wound, social nuisance etc. Among the mites, *Demodex* sp., *Sarcoptes scabiei* var. *canis* and *Otodectes cyonotes* are very common in dogs. Demodectic mange is considered as one the most common skin disease of dogs. The causative agent of canine demodicosis in dogs is *Demodex canis*, however, it can also be caused by *D. injai* (a large bodied mite) and *D. cornei* (a short bodied mite) as reported by Tater and Patterson (2008). Therefore, parasitic diseases of dogs are now become an issue of concern worldwide as they not only have detrimental effect on the animal's health, but also because of the associated zoonotic risk (Esenkaya *et al.*, 2018). Mite infestation in dogs have been reported in dogs in several states in India (Ballari *et al.*, 2009; Harkirat *et al.*, 2011; Chakraborty and Pradhan 2015; Swathi 2016). However, no systematic study on mite infestation in dogs has been conducted yet in the North-eastern India. Therefore, the present study was designed to explore the prevalence and identify mite infestation in dogs in and around Guwahati, Assam, India.

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MATERIALS AND METHODS

The present work was carried out in and around the Guwahati city, Assam, India. The study was conducted for one calendar year starting from March, 2019 to February, 2020. All the laboratory works were conducted at the Department of Parasitology, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam, India.

Collection of samples

A cross sectional study was conducted on 582 dogs of different breeds, age groups (below 1 year and above 1 year of age), sex and different categories (329 stray dogs, 166 pet dogs and 87 working dogs) to determine the prevalence and identification of *Demodex* mites in the study area. The dogs suffering from various dermatological problems with the history of either alopecia, itching, scales, crusts, papules or come dones were selected for taking skin scraping. The skin scraping was taken from at least two sites with adequate depth and peripheral edge. For collecting the skin scraping, hair around the lesions was clipped gently. The skin was grabbed between the thumb and forefinger and was scraped in the same direction several times with the help of a blunt scalpel dipped in liquid paraffin. Scraping

was continued until there was slight oozing of blood from dermal capillaries. Inclusion of superficial dry crusts, hairs etc. were avoided as much as possible. Scrapped material thus collected were transferred to clean vials containing phosphate buffer saline (PBS) or 70% alcohol and brought to the laboratory for detection of mite. The scrapped areas were applied with antibiotic ointments to prevent the occurrence of secondary bacterial infection.

Examination of skin scraping and detection of mite

The collected skin scraping materials were taken in a test tube with 10% potassium hydroxide (KOH) and heated as per routine laboratory procedure. Then the scraping materials were centrifuged at 1500 rpm for 3 minutes and supernatant was discarded. The sediments then taken on a clean glass slide, covered with cover slip and examined under compound microscope (X100 and X400 magnification) for detection of mite. Mites were identified following the procedure described by Soulsby (1982).

Morphometry of *Demodex* sp. mites

The morphometry of the *Demodex* sp. mites was done as per the procedure described by Fatima (2016) with slight modification. Correction factor for the microscope was measured first with the help of ocular and stage micrometer. Smears of processed skin scrapings of the dogs were used for measurements of the mites. Positive scraping material for *Demodex* sp. was suspended in a few drops of glycerine on a microscopic slide, a cover slip was applied and the preparation was examined under low power and then high power (10X, 40X) of microscope. Laboratory calibrated ocular and stage micrometer under compound microscope was used to measure the total mean body length and width, length of gnathosoma, podosoma and opisthosoma of the mite.

Extraction of DNA and molecular detection of *Demodex* sp.

From the positive skin scraping samples, three representative samples were selected for molecular detection of *Demodex* sp. Total DNA extraction from *Demodex* sp. positive skin scraping sample was done by using the DNeasy Blood and Tissue kit (Qiagen®) as per manufacturer's protocol. Quantification of the extracted DNA was done by nanodrop spectrophotometer (Eppendorf BioSpectrometer) and integrity was checked by agarose gel electrophoresis. Extracted DNA was kept at -20° C, until further use. The PCR was performed following the method of Sastre *et al.* (2012) with minor modification. Amplification was carried out using the *Demodex* genus specific primer pairs: Forward 52-GTA TTT TGA CTG TGC TAA GGY AGC-32 and Reverse 52-CAA AAG CCA ACA TCG AGG-32 for amplification of a 338 bp 16S rDNA gene fragment. The PCR amplification was performed using Dream Taq Master mix (Thermo scientific) in a 20 µl reaction mixture containing 2 µl (29.7 µg/mL) of DNA template and 1 µl (10 pmol/µl) of each primer. The PCR amplification was performed at 94° C for 10 minute for an initial denaturation, followed by 40 cycles of 30 sec denaturation at 94° C, annealing of 30 sec at 57° C and an extension of 30 sec at 72° C. Final extension was done at 72° C for 10 minute.

Statistical analysis

Results were expressed as the percentage. A difference with value $p<0.05$ was considered statistically significant. Chi-square test was performed to determine presence or absence of significant difference in parameters among the different groups using the Statistical Package for Social Sciences, Version 17.0.1 software (SPSS Inc., Chicago, IL, USA).

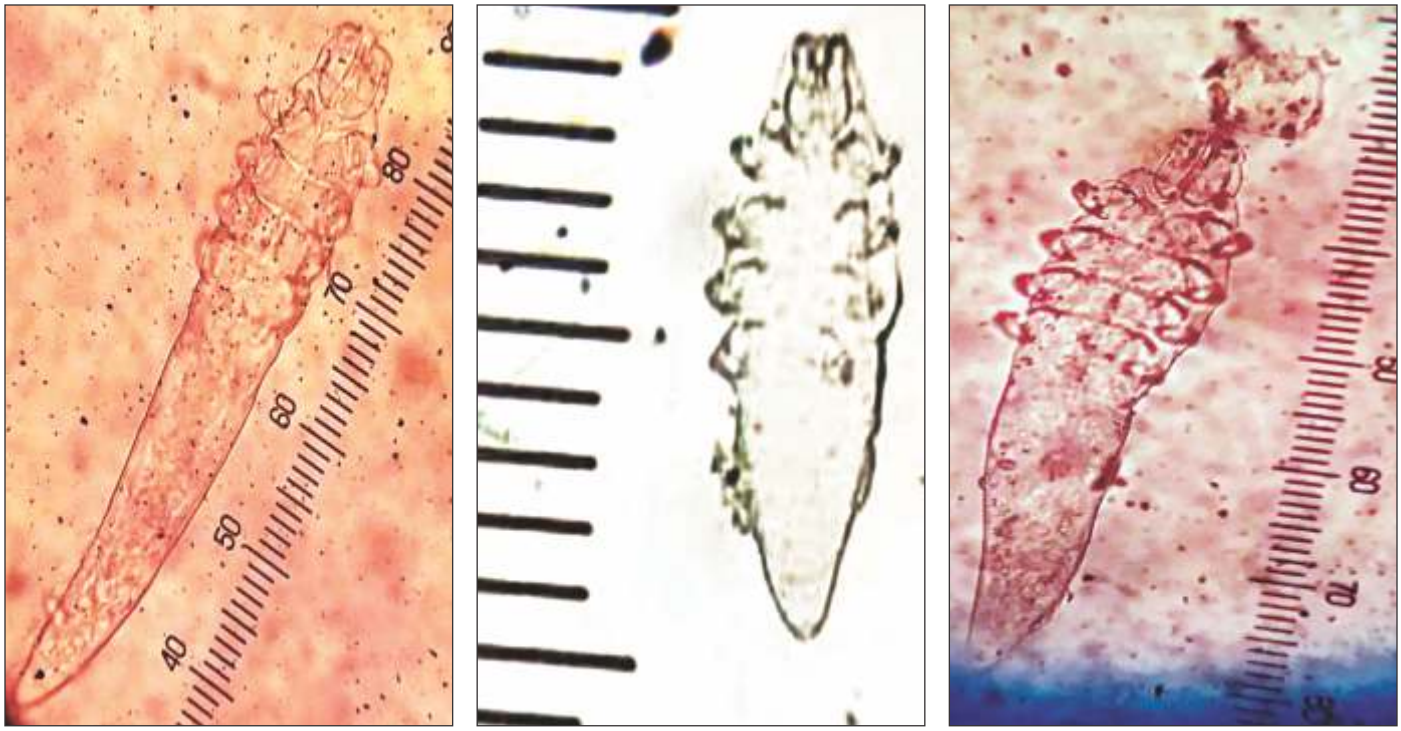
RESULTS AND DISCUSSION

Prevalence of *Demodex* infestation

In this study, out of 582 dogs examined, 115 were found positive for demodicosis with an overall prevalence of 19.75%. This reflected that the *Demodex* mite infestations were quite common in dogs in the study area. Our observations correlate with the findings of Ballari *et al.* (2009) in Chennai 21.25 %, Harkirat *et al.* (2011) in Ludhiana 19.40% and Swathi (2016) 20.54% in Hyderabad. However, the present percent prevalence was found more in comparison to the findings of Chakraborty and Pradhan (2015) (3.04% Kolkata, India) and less in comparison to the findings of Solanki *et al.* (2007) (25.45% from Gujarat, India) and Shrestha *et al.* (2015) (29.1% from Kathmandu, Nepal). The variation of the present findings might be due to variations in the geographical localities, climatic condition, available veterinary services and difference in the samples collection method.

Highest prevalence was found in Labrador retriever (42.72%) and least in Spitz (4.16%) breeds of dog (Table 1). Statistically, the influence of breed on Demodex mite infestation was found significant ($p<0.05$). Highest prevalence was found in Labrador retriever (42.72%), which was similar to that reported by Chakraborty and Pradhan (2015). Solanki *et al.* (2007) stated that pure breeds are more susceptible than crossbred dogs, though they observed highest prevalence in German shepherd (30.43%). However, Bindari *et al.* (2012) and Shrestha *et al.* (2015) reported higher prevalence of demodicosis in mongrels.

Sex-wise, the prevalence of demodicosis was more in males (23.02 %) than in female dogs (16.49 %) (Table 2). Statistically, the influence of sex on *Demodex* mite infestation was found significant ($p<0.05$). The results were similar to Ali *et al.* (2011), Swathi *et al.* (2016), Fatima *et al.* (2017) and Kaya *et al.* (2017). In contrary, incidence of canine demodicosis was reported more in females than



Figs. 1-3. **(1)** *Demodex injai* (400X) Each smallest division = 5 µm; **(2)** *D. cornei* each smallest division = 15 µm; **(3)** *Demodex canis* (400X) Each smallest division = 5 µm

male dogs by Islam and co-workers (2013). Higher prevalence of mite infestation in male dogs might be due to some hormonal influences mainly due to elevated plasma testosterone level (Roberts *et al.*, 2004). The aggressive and wondering habit of male dogs were responsible for more demodicosis occurrence among them (Lahkar *et al.*, 2005).

Age-wise prevalence of demodicosis in the present study was highest (32.74%) in young group (74/226) than in adult (41/356) group (11.51%) (Table 3). The influence of age on prevalence of Demodex mite infestation was found statistically significant ($p<0.05$). The findings were similar to various earlier studies conducted by Solanki *et al.* (2007) and Islam *et al.* (2013) who also reported higher prevalence of demodicosis in dogs of below 1 year of age. In contrary, Ahmed (2012) reported highest *Demodex* infection in dogs of more than 2 years of age. The higher prevalence of demodicosis in younger age group might be due to lack of immunity against different skin parasitic infestations (Kumar *et al.*, 2006). Shrestha *et al.* (2015) also opined that demodicosis is an immune-deficient disease and higher prevalence of demodicosis in lower age group of dog might be due to their low resistance than the adult. In young animals, mainly endo-parasiticism, malnutrition and debilitation lead to immune-compromised state which favours the mite to proliferate in the skin (Mueller, 2011).

Season-wise, highest prevalence of demodicosis was recorded in pre-monsoon (25.30%) followed by winter (21.58%), post-monsoon (16.51%) and monsoon (15.33%)

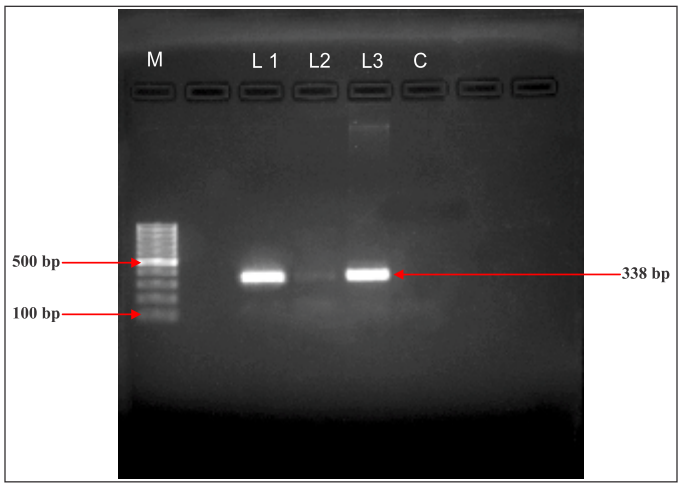


Fig. 4. Agarose gel (1.5%) showing PCR amplicon of 338 bp DNA fragment specific for *Demodex* sp. Lane L1, L2 &L3: *Demodex* positive skin scraping samples; Lane C: NTC; Lane M: DNA ladder (100 bp)

season (Table 4). Statistically, the influence of seasons on *Demodex* mite infestation was insignificant ($p>0.05$). Lahkar *et al.* (2005) found highest prevalence of demodicosis in monsoon season. Tsai *et al.* (2011) found highest prevalence in winter (12.5%). Kumar *et al.* (2018) found highest prevalence in summer season (25.42%). The high prevalence in winter may be because the temperature falls and all the dogs prefer to remain together in close contact of each other enhancing the possibility of transfer of mite from infected to healthy dog at ease.

Category-wise, prevalence of demodicosis was

highest in stray dogs (35.54%) followed by working dogs (21.83%) and pet dogs (11.24%) (Table 5). Statistically, the influence of different categories of dogs on *Demodex* mite infestation was found significant ($p<0.05$). Shrestha *et al.* (2015) also found prevalence rate was more among the free roaming dogs. Ananda *et al.* (2016) also found more infestation in stray dogs. The highest prevalence of the mite species observed in stray dogs during the present study might be due to the fact that stray dogs mostly suffer from malnutrition, endoparasitism, debility, total absence of any care and management as well as stress which act as predisposing factors causing diseases, leading to higher prevalence (Mueller, 2004).

Morphometry of *Demodex* mites using micrometers (Micrometry)

Morphometrically, three different species under the genus *Demodex* could be recorded where the highest mean length was recorded in *Demodex injai* followed by *Demodex canis* and least was found in *Demodex cornei*. The detailed morphological and morphometric descriptions are presented in Table 6. (Figs. 1-3). The morphological and morphometric values obtained in the present study for *Demodex injai* were similar to that of reported by Izdebska (2010), Izdebska and Fryderyk (2011) and Swathi *et al.* (2016). Whereas in case of *Demodex canis*, morphological and morphometric values obtained were similar to that of reported by Izdebska (2010), Sakulploy and Sangvaranond (2010), López *et al.* (2011), Sivajothi *et al.* (2013) and Swathi *et al.* (2016). Further, in case of *Demodex cornei*, morphological and morphometric values were similar to that of reported by Sakulploy and Sangvaranond (2010), Taiju (2010), López *et al.* (2011), Sivajothi *et al.* (2013) and Swathi *et al.* (2016).

Molecular detection of *Demodex* sp.

For molecular study, three representatives of *Demodex* positive skin scrapings were utilized for extraction of DNA, which were marked as L1, L2 and L3. The 16S rDNA gene fragment was targeted for molecular identification of *Demodex* mite. Out of 3 samples, the amplicons numbering L1 and L3 showed distinct band and L2 showed little light *Demodex genus* specific band at 338 bp when compared with the marker. The results were in agreement with Satre *et al.* (2012) (Fig. 4).

Table 1. Breed-wise prevalence of Demodicosis in dogs

Breeds	No. examined	No. positive for *Demodex* sp.	Prevalence (%)
Mongrel	252	28	11.11
German shepherd	69	19	27.53
Labrador retriever	110	47	42.72
Pug	45	8	17.77
Spitz	48	2	4.16
Golden retriever	18	4	22.22
Doberman pinscher	16	4	25.00
Cocker spaniel	10	1	10.00
Pomeranian	08	1	12.50
Bull mastiff	06	1	16.66
Total	582	115	19.75

Table 2. Sex-wise prevalence of demodicosis in dogs

Sex	No. of dogs examined	No. of dogs positive	Prevalence (%)
Male	291	67	23.02
Female	291	48	16.49
Total	582	115	19.75

Table 3. Age-wise prevalence of demodicosis in dogs

Age Group	No. of dogs examined	No. of positive	Prevalence (%)
Young (<1 year)	226	74	32.74
Adult (>1 year)	356	41	11.51
Total	582	115	19.75

Table 4. Season-wise prevalence of demodicosis in dogs

Season	No. examined	No. positive	Prevalence (%)
Pre monsoon	136	34	25.30
Monsoon	226	35	15.33
Post monsoon	90	17	16.51
Winter	130	29	21.58
Total	582	115	19.75

Table 5. Category-wise prevalence of demodicosis in dogs

Categories	No. examined	No. positive	Prevalence (%)
Pet dogs	329	37	11.24
Stray dogs	166	59	35.54
Working dogs	87	19	21.83
Total	582	115	19.75

CONCLUSION

Considerably high prevalence of mite infestation in dogs was observed in and around Guwahati, Assam, India. *Demodex* prevalence was recorded throughout the year in with highest prevalence recorded in pre-monsoon season. The breed, sex, age and category wise study showed the highest prevalence of *Demodex* infestation in Labrador retriever breed, in male dogs, in dogs of below 1 year of age and in stray dogs, respectively. Three species of *Demodex* namely *Demodex injai*, *Demodex canis* and *Demodex cornei* could be recorded in the present study based on morphometry of *Demodex* mites. To best of our knowledge, our study reports the molecular detection of *Demodex* in dogs for the first time from the North east region of India.

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Table 6. Morphometric descriptions of the three *Demodex* sp. identified

Name of the mite	Length				Total body width (µm) (Range)
	Gnathosoma (µm) (Range)	Podosoma (µm) (Range)	Opisthosoma (µm) (Range)	Total body length (µm) (Range)	
D. canis	27.61 (22.5-37.5)	65.38 (60-71.5)	125.96 (75.5-149.5)	218.96 (173-242.5)	36.5 (34.4-42.6)
D. cornei	24.14 (22.5-25.5)	54.57 (48-61.5)	64.14 (60.5-70.5)	142.85 (134.5-157.5)	38.5 (36.5-43.5)
D. injai	33.93 (29-42.5)	78.5 (72-89.5)	164.93 (148.5-182)	277.12 (272-290.5)	44.5 (35.6-46.8)

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